Supporting information

Lysine deacetylase substrate selectivity: a dynamic ionic interaction specific to KDAC8

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Table S1. Specific activity values of peptides in low ionic strength buffer plotted as normalized values

Enzyme	Peptide	Specific activity (s^{-1})	Activity (pmol s^{-1})†
KDAC8	FRK^{ac}RW	0.035 ± 0.007	
KDAC8	FRK^{ac}RW	0.025 ± 0.002*	
KDAC8	ISK^{ac}FD	0.0040 ± 0.0009	
KDAC8	SLK^{ac}FG	0.0084 ± 0.0016	
KDAC8	FRK^{ac}AW	0.034 ± 0.004	
KDAC8	FAK^{ac}RW	0.0039 ± 0.0007	
KDAC8	ARK^{ac}AA	0.0028 ± 0.0009	
KDAC8	AAK^{ac}RA	0.0000 ± 0.0002	
KDAC8	FKK^{ac}RW	0.016 ± 0.003*	
KDAC8	FQK^{ac}RW	0.009 ± 0.003	
KDAC8	FEK^{ac}RW	0.0008 ± 0.0005	
KDAC8 D101E	FRK^{ac}RW	0.029 ± 0.004	
KDAC8 D101N	FRK^{ac}RW	0.0000 ± 0.0004	
KDAC8 D101A	FRK^{ac}RW	0.0000 ± 0.0005	
KDAC8 D101R	FRK^{ac}RW	0.0001 ± 0.0007	
KDAC8 D101E	FKK^{ac}RW	0.017 ± 0.007*	
KDAC8 D101E	FQK^{ac}RW	0.0057 ± 0.0011	
KDAC8 D101E	FAK^{ac}RW	0.0052 ± 0.0007	
KDAC8 D101E	FEK^{ac}RW	0.0009 ± 0.0003	
KDAC1	FRK^{ac}RW		0.32 ± 0.03
KDAC1	FRK^{ac}RW		0.030 ± 0.015*
KDAC1	FKK^{ac}RW		0.036 ± 0.018*
KDAC1	FQK^{ac}RW		0.30 ± 0.03
KDAC1	FAK^{ac}RW		0.16 ± 0.02
KDAC1	FEK^{ac}RW		0.033 ± 0.005
KDAC1	FRK^{ac}AW		0.22 ± 0.05
KDAC6	FRK^{ac}RW	0.024 ± 0.009	
KDAC6	FRK^{ac}RW	0.029 ± 0.004*	
KDAC6	FKK^{ac}RW	0.027 ± 0.004*	
KDAC6	FQK^{ac}RW	0.015 ± 0.009	
KDAC6	FAK^{ac}RW	0.0179 ± 0.0019	
KDAC6	FEK^{ac}RW	0.031 ± 0.008	
KDAC6	FRK^{ac}AW	0.0241 ± 0.0015	

* indicates measured using mass spectrometry assay.

† KDAC1 is reported as raw activity because the commercial sample was of low purity.

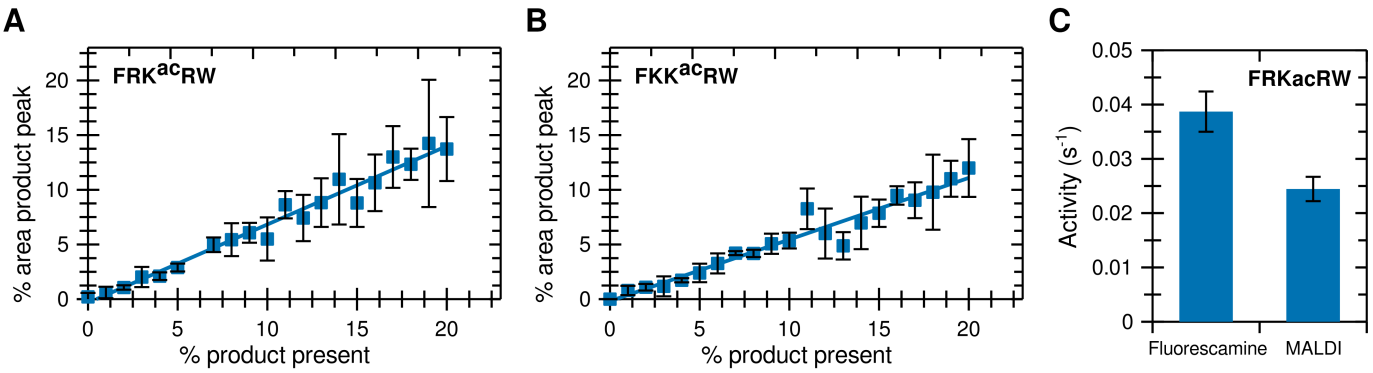

Figure S1. MALDI-TOF assay to measure specific activity of lysine deacetylases with peptide substrates. (A) Standard curve of peak area ratios from mass spectrometry of defined ratios of product peptide (FRKRW) and substrate peptide (FRK^{ac}RW). Error bars represent standard deviations (n≥3). The line represents the linear fit. (B) Standard curve of peak area ratios from mass spectrometry of defined ratios of product peptide (FKKRW) and substrate peptide (FKK^{ac}RW). Data is represented as in panel A. (C) KDAC8 was reacted with FRK^{ac}RW. Each reaction replicate was assayed by both the fluorescamine assay as previously reported and the MALDI-TOF assay described here.[22] Error bars represent standard deviations (n=4). The difference in activity was not statistically significant using a paired t-test ($p > 0.05$).

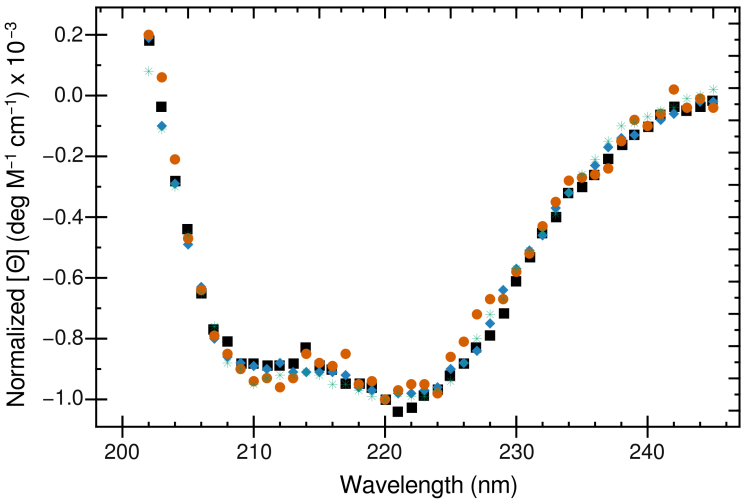

Figure S2. Circular dichroism spectra of KDAC8 variants do not indicate significant structural differences. Circular dichroism was performed with wild-type KDAC8 (black squares), KDAC8 D101N (blue diamonds), KDAC8 D101A (red circles) and KDAC8 D101R (green stars).